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## **CLAIMS**

**#1**

**The publication of my UK patent entitled 'A surgical-medical dressing for the treatment of body burns and for wound healing' on February 13<sup>th</sup>, 2002 will arouse academic and commercial interest in HUVECs CM as well as my submitted 2001 paper.**

**If this dressing is to be used to treat a burns patient, it is envisaged that the whole dressing, will be enclosed in a gel-like substance, to facilitate easy handling by the attending physician. This gel-like substance will dissolve soon after being applied, i.e. contact with the patient's skin.**

**If this dressing is to be used in the treatment of a wound, it will be placed in the cavity of the wound and not removed. It is envisaged that several 'strips' of this dressing would be used depending on the size and depth of the wound.**

**It is envisaged that allogenic neonatal or infant keratinocytes (sparsely seeded) will be grown on the upper/outer layer of the dressing, if required for an 'occlusive dressing' use instead of an allograft/autograft.**

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**Claim #2**

**Human vein endothelial cells (HUVECs) conditioned  
medium (CM) -----HUVECs CM.**

**The first scientific report of the tissue culture of  
HUVECs was made by Jaffe et al. in 1973. This report  
described the perfusion of an umbilical cord with an  
enzyme, collagenase and the growth to confluence of  
HUVECs. Basic fibroblast growth factor (b-FGF) or  
commercially available endothelial cell growth  
supplements (ECGS) are added to the nutrient solution,  
(called the medium) to ensure that confluency is  
attained. There are scientific reports by Gospodarowicz  
et al. 1980a,b proving that assertion.**

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Little attention has been paid to the conditioned medium (CM) which is obtained after the nutrient medium is left in contact with the proliferating cells for periods of time which may vary from 2-3 days or longer. When the medium is changed, the conditioned medium is always discarded and an aliquot of fresh medium added.

In 1992, I published the first paper that showed a linkage between the extracellular secretions of HUVECs and human aortic endothelial cells. HUVECs were grown to post confluency (2 days after normal confluence) and the cell layer was detached with 5mM EDTA (ethylene diamine tetra-acetic acid) leaving behind the sub-endothelial matrix (the so-called extracellular matrix

**(ECM)) intact. (The detached cells were reseeded onto a fresh substrate and re-grown time and again.)**

**Human aortic endothelial cells were obtained by mechanical scraping the intimal lining of a segment of human aorta, and these endothelial cells were grown to confluence on the HUVECs ECM, obtained as described above. A postulate was proposed that ECMs had a common phenotype.**

**In 2002, in a submitted paper to 'The International Journal of Experimental Pathology', I illustrated the fact that HUVECs CM may be used as a culture medium for human dermal microvascular endothelial cells (HDMECs), dermal fibroblasts, and epidermal**

**keratinocytes, because of related extracellular secretions (see Table I above). The CM may be pre-screened by bacteriological and other tests before use as required.**

**DISPASE, a neutral protease is used to detach epithelial sheets (epidermal keratinocytes are grown in sheets in a tissue culture laboratory) which are used clinically as cultured epithelial autografts (CEAs) in the management of body burns.**

**Because CEAs are manufactured using Dispase, the 'take' of the graft is impaired (Rennekampff et al. 1996).**

**The scientific paper describing Dispase was published in 1992. The title of the paper was: Dispase, a neutral**

protease from *Bacillus polymxa*, is a powerful

fibronectinase and Type IV collagenase.

HUVECs secrete into their conditioned medium (CM),

Type IV procollagen, fibronectin and thrombospondin

which makes the HUVECs CM a natural neutralizing

agent for Dispace.

Cascade Biologics, Inc. (Portland, OR), to name just one

company in this line of business, sell all types of tissue

culture media, cell types, ECGS etc. but not HUVECs

CM. Interestingly, they sell pooled HUVECs, from more

than one umbilical cord. The resultant ECM will not be

pure as an original from one umbilical cord.

Scientific references:



**Jaffe E.A., Nachman R.L., Becker C.G. & Minick C. (1973)**

**Culture of human endothelial cells derived from  
umbilical veins. Identification by morphologic and  
immunologic criteria. J. Clin. Invest. 52, 2745-2756.**

**Gospodarowicz D., Delgado D. & Vlodavsky I. (1980a)**

**Permissive effect of the extracellular matrix on cell  
proliferation in vitro. Proc. Natl. Acad. Sci. USA 77, 4094-  
4098.**

**Gospodarowicz D. & Ili C. (1980b) Extracellular matrix  
and control of proliferation of vascular endothelial cells.  
J. Clin. Invest. 65(6), 351-64.**

**Solomon D.E. (1992) The seeding of human aortic  
endothelial cells on the extra-cellular matrix of human**

**umbilical vein endothelial cells. Int. J. Exp. Path. 73, 491-501.**

**Stenn K.S., Link R., Moellmann G., Madri J. & Kuklinska E. (1989) Dispase, a neutral protease from Bacillus polymyxa, is a powerful fibronectinase and type IV collagenase. J. Invest. Dermatol. 93(2), 287-290.**

**Rennekampff H.O., Kiessig V. & Hansbrough J.F. (1996) Research Review-Current concepts in the development of cultured skin replacements. J. Surg. Res. 62, 288-295.**

**Solomon D.E. (2002) Submitted Dec. 2001--- Int. J. Exp. Pathol. The inter-relationships between human endothelial cells, dermal fibroblasts and epidermal keratinocytes extracellular secretions, and their connection to wound healing.**

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## **Claims**

### **# 3**

#### **By-product**

**A can spray of cornstarch and oil of cloves mixture.**

**Presently, a product called 'Water.Jel' (Carlstadt, NJ) is used by City of Miami paramedics in onsite, first aid treatment of burn victims. My suggestion was initially an antibiotic-100% cornstarch spray in a durable container like a can spray. Enquiries have revealed that 'Water.Jel', a soothing wrap, suffers from fungi infection, and puncture of its packaging. Instead of using an antibiotic, and circumventing FDA approval, a mixture of oil of cloves and 100% cornstarch, packaged in a spray can is contemplated as a by-product.**

**Why cornstarch? It is a white granular carbohydrate occurring in the endosperm of corn kernel. It enjoys wide use as a major constituent of talcum body powder, in domestic cooking as a thickener, (a binding agent), for soups and stews and in the laundry product, spray starch. Cornstarch is used to soothe sunburn, for the relief of diaper rash, prickly heat, and itchy, irritated skin.**

**On arrival on hospital property, this white coating could be easily removed by salving the affected area.**

**Alternatively, if maggots are to be used, they can be applied without salving. Anything that can stem tissue bleeding, loss of oozing wound fluid, and provide some small measure of pain relief, at the site of the incident, will be an improvement on 'Water.Jel'.**

**This proposed spray should compromise the blood coagulation cascade, and the release of cellular growth**

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**factors e.g. PDGF and alpha-thrombin, which cause recruitment to the wound bed and both the proliferation and migration of fibroblasts.**

**It will also approximate an occlusive dressing, because there will be a vapor barrier. Minutes of the so-called 'golden hour' might be used beneficially, or even saved.**

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